Role of Relish/NF-kB Apoptosis Pathway in Amyloid-beta42 Mediated Neurodegeneration in Alzheimer's Disease

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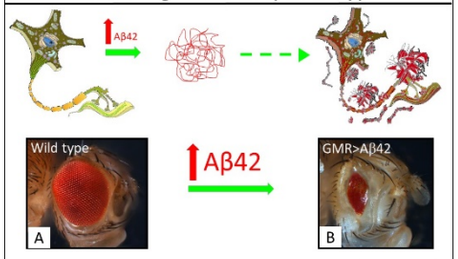
Abstract

Alzheimer's disease (AD) is a neurodegenerative disease which affects the mental functions of the patients. This disorder progresses with age and does not have a cure to-date. One of the reasons for the manifestation of AD is the accumulation of amyloid-beta-42 (Aβ42) proteins. In our study, we have used *Drosophila* as our model organism (as 75% of the genetic machinery is conserved between flies and humans), and have developed a model where when human Aβ42 is misexpressed in the differentiating eye, triggers cell death in the retinal neurons. We have also identified a soy-based anti-inflammatory protein, Lunasin, which can block Aβ42 mediated cell death by downregulating the NFkB pathway (which lead to translation of apoptotic proteins of jun-N Terminal Kinase (JNK) pathway). In order to discern the exact mechanism by which Lunasin prevents neuronal cell death (caused by the accumulation of Aβ42 proteins), we have developed transgenic flies which can produce human Aβ42 and Aβ42+Lunasin in the *Drosophila* eye. Our hypothesis states that manipulating the Relish protein complex of the Imd-NFKB pathway could lead to activity variation in JNK pathway in Aβ42+Lunasin flies. To test our hypothesis, we used GAL4/UAS system genetic technique and misexpressed Relish and Relish^{RNAi} in human Aβ42, Aβ42+Lunasin background and checked for the resultant phenotypes in (1) larval eye imaginal discs and in (2) the adult eyes. Our data showed that downregulating Relish leads to eye suppression phenotypes, which suggests that the Imd-NFKB pathway plays a positive role in Lunasin's ability to mitigate the neuronal cell death cause by the accumulation of Aβ42 plaques. These studies have significant bearing on the use of NFKB members as biomarkers or druggable targets and generate new insights into the mechanism by which Aβ42 mediated neurodegeneration cell death can be blocked in the future.

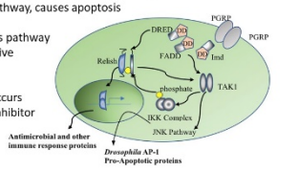
Our fly model exhibits neurodegenerative phenotype



Sarkar et al., 2016

Imd Relish Pathway

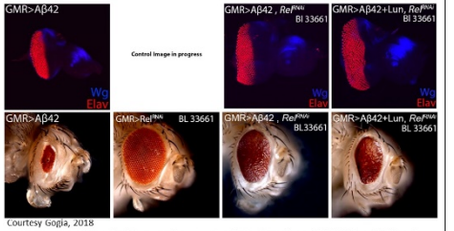
- Imd - Immune deficiency pathway, causes apoptosis
- The primary response of this pathway is to respond to gram-negative bacteria and inflammation.
- Branching in the pathway occurs when TAK1 activates both Inhibitor of Kappa-B kinase (IKK) and c-Jun N-terminal kinase (JNK) pathway.



- Activation of the JNK pathway correlates with apoptosis.
- The DRED protein and the IKK complex cleave Relish complex, allowing it to translocate to nucleus where it binds as a dimer to Relish response elements.
- The products of DNA binding are antimicrobial and immune response proteins.
- Termination of Imd and JNK pathway can be induced by degradation of TAK1.

(Hetru et al., 2009)

Down-regulating *Relish* interferes with lunasin's ability to rescue Aβ42 phenotype

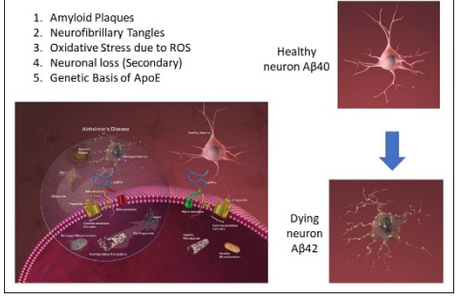


Courtesy Gogia, 2018

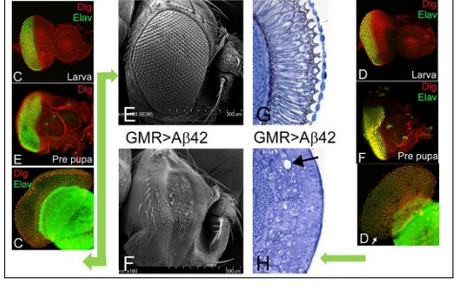
- Down-regulating *Relish* caused a suppression phenotype in both the Aβ42 and Aβ42+Lunasin flies
- Absence of *Relish* did interfere with the save phenotype of Aβ42+Lunasin

Alzheimer's Disease

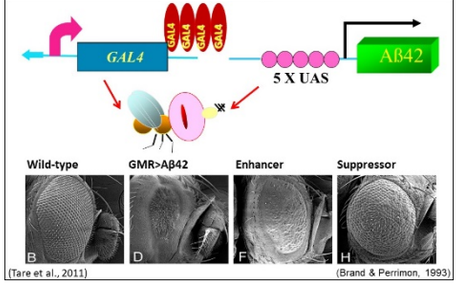
1. Amyloid Plaques
2. Neurofibrillary Tangles
3. Oxidative Stress due to ROS
4. Neuronal loss (Secondary)
5. Genetic Basis of ApoE



Drosophila eye model to study Aβ42 mediated neurodegeneration

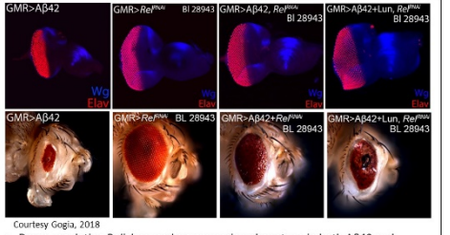


Gain-of-function approach: GAL4/UAS system



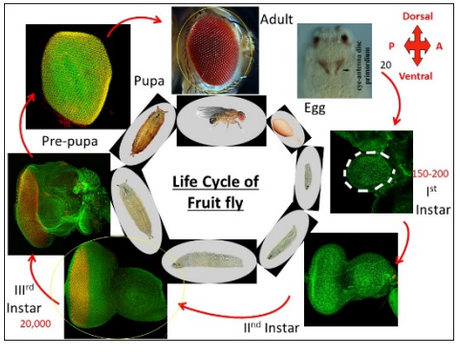
(Tare et al., 2011) (Brand & Perrimon, 1993)

Down-regulating *Relish* interferes with lunasin's ability to rescue Aβ42 phenotype



Courtesy Gogia, 2018

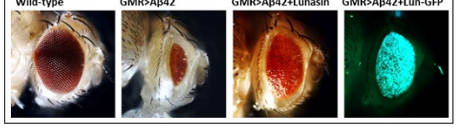
- Down-regulating *Relish* caused a suppression phenotype in both Aβ42 and Aβ42+Lunasin flies
- Absence of *Relish* did interfere with the save phenotype of Aβ42+Lunasin



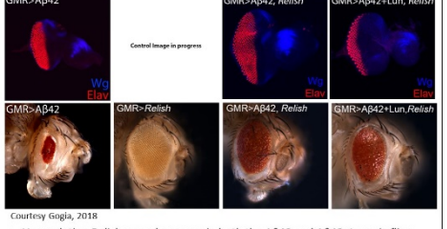
Soybean protein: Lunasin

- Lunasin (Lun), is a 43 amino acid long Soy bean protein.
- The carboxyl end contains a poly-aspartic (8 A.A long) tail that is used for chromatin binding.
- Gly-Arg-Gly(RGD) motif is responsible for internalization of the chain into the cell nucleus.

- **Applications:**
- Cancer research related to tumor suppression.
- Heart disease research links Lunasin to be an anti-inflammatory.



Upregulating Relish doesn't interfere with lunasin's ability to rescue Aβ42 phenotype



Courtesy Gogia, 2018

- Upregulating *Relish* caused a rescue in both the Aβ42 and Aβ42+Lunasin flies
- *Relish* did not interfere with the save phenotype of Aβ42+Lunasin

Conclusions and Future Directions

Conclusions

- Lunasin has shown potential to induce a rescue phenotype in the Aβ42 model.
- Upregulating the Relish protein complex along with Lunasin showed a rescue phenotype as well.
- Both Relish RNAi crosses created detrimental effects when crossed with Lunasin Aβ42 flies.
- These observations lead us to believe that the Relish protein complex and the Imd Pathway are involved in Lunasin's ability to induce a rescue Aβ42 phenotype.

Future Directions

- Identify other parts of Imd pathway involved in this process.
- To check the effect on downstream targets: Diptericin and Dredd proteins, using both GOF and LOF studies.
- Identify interactions with other immune response pathways, such as Toll receptor pathway.
- Determine the mechanism of action by which rescue and suppression phenotypes are established.

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